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13. ABSTRACT ***(Maximum 200 Words)***

Imaging procedures play an important role in the current management of patients with prostate cancer. Despite advances in many of these methodologies, improvements are still needed, especially in the area of Nuclear Medicine. The radioiodinated phospholipid ether analogs being investigated under this grant represent a new class of radiopharmaceutical, which has provided excellent images of prostate tumors in animal models. Two of the newer analogs, namely NM-404 and NM-412, were found to be significantly superior to the prototype agent, NM-324 in both the rat and mouse tumor models, while avoiding the high first pass clearance by the liver displayed by NM-324. We are actively pursuing FDA approval of an IND to initiate pharmacokinetic, distribution and elimination studies of radioiodinated NM-404 in prostate cancer patients.

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5. INTRODUCTION:

Our laboratories have prepared and evaluated a number of radioiodinated phospholipid ether (PLE) analogs as candidates for the noninvasive imaging of human cancers. Several of these agents have been shown to selectively accumulate in a variety of animal tumors as well as human tumor xenografts. Moreover, preliminary results with one of the early candidates (NM-324) in cancer patients confirmed the ability of such agents to be taken up and retained by certain cancers. On the other hand, follow up studies in Copenhagen rats bearing Dunning R3327 prostate tumors (MAT-LyLu) and in SCID mice with human PC-3 xenografts showed NM-324 to be a poor candidate for visualizing prostate cancer. Therefore, a major effort was undertaken to develop agents better suited to imaging prostate cancer. Two of these newer analogs, namely NM-404 and NM-412, were found to be significantly superior to NM-324 in both the rat and mouse tumor models. Furthermore, lymphatic metastases were clearly delineated in the Dunning rat model. We believe NM-404 and NM-412 are excellent candidates for the diagnosis of prostate cancer and associated metastatic lesions and thus warrant further preclinical and subsequent clinical evaluation.

6. BODY:

Based upon reports that human tumor tissue contains significantly higher levels of phospholipid ether (PLE) than adjacent normal tissue, our laboratory designed and synthesized a number of radioiodinated PLE analogs as potential tumor imaging agents. Several of these agents showed a striking ability to be taken up and retained by a variety of animal tumors and human tumor xenografts. In an effort to establish the relevance of our animal models to the human situation, one candidate (NM-324) was selected for a preliminary evaluation in cancer patients. This study showed that NM-324 was capable of imaging tumors in patients, but high first pass clearance by the liver severely compromised its clinical utility as a diagnostic radiopharmaceutical. However, this study demonstrated that our animal models were appropriate for the identification of clinical candidates. Therefore, the design of second-generation candidates was focused on those that would possess a longer plasma half-life and /or more rapid metabolic clearance by the liver and other non-target tissues. This led to the identification of two agents, namely NM-404 and NM-412.

Thus our overall hypothesis is that appropriately radioiodinated NM-404 or NM-412 will be effective tumor imaging agents in humans. To prove this, their localization must first be shown to be effective in animals, toxicity data must be obtained in animals to show that the agents are safe, dosimetry predictions based on the biodistribution results in animals must be performed, and finally the unlabeled and radiolabeled agent must evaluated in cancer patients.

Last year's report indicated that all of the preclinical work on NM-404 and most of that for NM-412 had been completed. This year's effort thus focused on completion of the preclinical work-up on NM-412 and preparation for the clinical evaluation of NM-404. The biodistribution of radioiodinated NM-412 in normal, male Sprague-Dawley rats at various time points was completed and the results shown in Table 1. From this data our health physicist, Dr. Zealand, was able to calculate the radiation dose to human tissues. Table 2 compares NM-412 results with those for NM-324 and NM-404. Based on this data, Dr. Zasadny concluded that studies could be undertaken in cancer patients with a maximum dose of 3 mCi following thyroid blockade with Lugol's solution (SSKI).

Biodistribution of NM-412 in male SCID mice bearing human PC-3 prostate tumor xenografts ($1x10^6$ cells, i.m.) following i.v. injection of ^{125}I-NM-412 revealed that tumor tissue accumulated the agent in a time-dependent manner while other tissues much more rapidly accumulated and eliminated the agent. Within five days of injection, the target to non-target ratios of NM-412 exceeded 4.5 for all but three tissues (Table 3). Gamma camera imaging of human PC-3 prostate tumor xenografts in male SCID mice following i.v. injection of ^{125}I-NM-412 demonstrated that the radiotracer was efficiently eliminated from normal tissues while being selectively retained in tumor tissue located in the thigh region at time points similar to those sampled in the biodistribution studies. (see Appendix 1).

Continued efforts by Dr. Van Dort to improve our radioiodination procedure led to a new process that afforded a significant increase in the specific activity of the products. Because of this discovery, both NM-404 and NM-412 have been shown to be safe in two animal species at a dose level over 12,000 times the anticipated human imaging dose. Dr. Paul Kosyniak at the Center for Toxicology at SUNY Buffalo conducted these toxicity studies, which were submitted to the FDA as part of our IND application (IND # 62,703).

TABLE 1. BIODISTRIBUTION OF ^{125}I-NM-412 IN 2% TWEEN 20/STERILE WATER IN MALE SPRAGUE-DAWLEY RATS FOLLOWING I.V. INJECTION.

NM-412 1 Day (n=4)	dpm/mg	% dose/gm ± SEM	% kg-does/gm ± SEM	% dose/organ ± SEM
Adrenal	122.649 ±9.922	0.939 ± 0.092	0.219 ± 0.020	0.056 ± 0.005
Blood	30.400 ±1.536	0.233 ± 0.017	0.054 ± 0.003	2.689 ± 0.169
Bone Marrow	50.994 ± 3.887	0.390 ± 0.036	0.091 ± 0.008	0.316 ± 0.027
Duodenum	46.064 ± 4.552	0.354 ± 0.044	0.082 ± 0.009	1.591 ± 0.174
Eye	5.468 ± 0.887	0.042 ± 0.008	0.010 ± 0.002	0.013 ± 0.002
Fat	18.999 ± 2.646	0.145 ± 0.021	0.034 ± 0.005	2.410 ± 0.360
Heart	21.540 ± 1.503	0.164 ± 0.012	0.038 ± 0.003	0.111 ± 0.008
Kidney	57.235 ± 3.774	0.438 ± 0.036	0.102 ± 0.008	0.778 ± 0.059
Liver	49.792 ± 2.917	0.380 ± 0.022	0.089 ± 0.006	3.880 ± 0.105
Lung	63.950 ± 7.836	0.487 ± 0.058	0.114 ± 0.014	0.639 ± 0.078
Muscle	10.650 ± 0.876	0.081 ± 0.008	0.019 ± 0.002	8.641 ± 0.697
Plasma	42.631 ± 2.541	0.326 ± 0.027	0.076 ± 0.005	2.073 ± 0.149
Prostate	20.251 ± 1.490	0.155 ± 0.014	0.036 ± 0.003	0.000 ± 0.000
Skin	27.142 ± 2.986	0.207 ± 0.023	0.049 ± 0.006	8.733 ± 1.008
Spleen	55.069 ± 2.844	0.420 ± 0.025	0.098 ± 0.006	0.293 ± 0.021
Testes	15.503 ± 1.356	0.119 ± 0.012	0.028 ± 0.003	0.000 ± 0.000
Thyroid	4624.799 ± 218.561	35.321 ± 2.015	8.262 ± 0.479	0.620 ± 0.036
Urinary Bladder	31.393 ± 3.050	0.241 ± 0.028	0.056 ± 0.006	0.000 ± 0.000
NM-412 3 Day (n=4)				
Adrenal	89.169 ±5.668	0.687 ± 0.030	0.172 ± 0.005	0.044 ± 0.001
Blood	12.081 ± 0.396	0.094 ± 0.004	0.023 ± 0.001	1.160 ± 0.063
Bone Marrow	31.589 ± 1.256	0.244 ± 0.011	0.061 ± 0.003	0.212 ± 0.009
Duodenum	22.852 ± 1.413	0.176 ± 0.009	0.044 ± 0.002	0.852 ± 0.048
Eye	4.429 ± 0.227	0.034 ± 0.001	0.009 ± 0.000	0.011 ± 0.001
Fat	20.378 ± 2.393	0.157 ± 0.018	0.039 ± 0.005	2.795 ± 0.349
Heart	9.448 ± 0.398	0.073 ± 0.004	0.018 ± 0.001	0.053 ± 0.003
Kidney	32.284 ± 0.844	0.249 ± 0.004	0.062 ± 0.002	0.474 ± 0.012
Liver	23.407 ± 0.637	0.181 ± 0.007	0.045 ± 0.002	1.843 ± 0.058
Lung	34.258 ± 0.999	0.265 ± 0.013	0.066 ± 0.003	0.372 ± 0.019
Muscle	5.213 ± 0.145	0.040 ± 0.002	0.010 ± 0.001	4.604 ± 0.257
Plasma	17.059 ± 0.787	0.132 ± 0.007	0.033 ± 0.002	0.899 ± 0.052
Prostate	13.141 ± 1.110	0.101 ± 0.007	0.025 ± 0.002	0.000 ± 0.000
Skin	21.706 ± 1.855	0.168 ± 0.015	0.042 ± 0.003	7.544 ± 0.621
Spleen	29.845 ± 1.874	0.230 ± 0.013	0.058 ± 0.003	0.127 ± 0.005
Testes	12.930 ± 0.385	0.100 ± 0.002	0.025 ± 0.000	0.000 ± 0.000
Thyroid	2896.927 ± 90.447	22.371 ± 0.307	5.598 ± 0.123	0.420 ± 0.009
Urinary Bladder	22.101 ± 1.349	0.170 ± 0.007	0.043 ± 0.002	0.000 ± 0.000

NM-412 5 Day (n=4)	dpm/mg	% dose/gm ± SEM	% kg-does/gm ± SEM	% dose/organ ± SEM
Adrenal	61.839 ± 5.512	0.446 ± 0.033	0.089 ± 0.006	0.023 ± 0.002
Blood	7.224 ± 1.305	0.052 ± 0.008	0.010 ± 0.002	0.511 ± 0.083
Bone Marrow	20.064 ± 1.238	0.145 ± 0.008	0.029 ± 0.001	0.100 ± 0.004
Duodenum	16.391 ± 1.940	0.118 ± 0.013	0.023 ± 0.002	0.453 ± 0.045
Eye	4.448 ± 0.625	0.032 ± 0.004	0.006 ± 0.001	0.008 ± 0.001
Fat	25.814 ± 1.983	0.187 ± 0.016	0.037 ± 0.003	2.644 ± 0.221
Heart	6.462 ± 0.443	0.047 ± 0.002	0.009 ± 0.000	0.027 ± 0.001
Kidney	25.904 ± 1.535	0.187 ± 0.008	0.037 ± 0.001	0.283 ± 0.010
Liver	22.883 ± 1.663	0.165 ± 0.009	0.033 ± 0.002	1.002 ± 0.059
Lung	24.734 ± 3.568	0.177 ± 0.022	0.035 ± 0.004	0.198 ± 0.025
Muscle	4.967 ± 0.976	0.036 ± 0.008	0.007 ± 0.002	3.308 ± 0.747
Plasma	8.581 ± 2.476	0.061 ± 0.016	0.012 ± 0.003	0.330 ± 0.091
Prostate	9.205 ± 0.897	0.067 ± 0.007	0.013 ± 0.001	0.000 ± 0.000
Skin	20.517 ± 0.638	0.148 ± 0.004	0.030 ± 0.001	5.335 ± 0.208
Spleen	22.204 ± 1.707	0.161 ± 0.012	0.032 ± 0.002	0.072 ± 0.003
Testes	12.848 ± 0.689	0.093 ± 0.003	0.018 ± 0.001	0.000 ± 0.000
Thyroid	3523.361 ± 240.666	25.537 ± 1.951	5.078 ± 0.315	0.381 ± 0.024
Urinary Bladder	15.425 ± 1.817	0.111 ± 0.011	0.022 ± 0.002	0.000 ± 0.000
NM-412 8 Day (n=4)				
Adrenal	29.256 ± 1.685	0.259 ± 0.017	0.070 ± 0.003	0.018 ± 0.001
Blood	1.798 ± 0.108	0.016 ± 0.001	0.004 ± 0.000	0.215 ± 0.014
Bone Marrow	6.721 ± 0.963	0.059 ± 0.009	0.016 ± 0.002	0.056 ± 0.007
Duodenum	4.901 ± 0.284	0.043 ± 0.002	0.004 ± 0.001	0.006 ± 0.001
Fat	22.953 ± 1.155	0.203 ± 0.009	0.055 ± 0.003	3.925 ± 0.244
Heart	1.943 ± 0.144	0.017 ± 0.001	0.005 ± 0.000	0.013 ± 0.001
Kidney	7.156 ± 0.619	0.063 ± 0.006	0.017 ± 0.001	0.130 ± 0.009
Liver	6.457 ± 0.207	0.057 ± 0.002	0.016 ± 0.001	0.588 ± 0.019
Lung	7.155 ± 0.909	0.063 ± 0.009	0.017 ± 0.002	0.096 ± 0.011
Muscle	1.201 ± 0.160	0.011 ± 0.002	0.003 ± 0.000	1.311 ± 0.159
Plasma	2.328 ± 0.114	0.021 ± 0.001	0.006 ± 0.000	0.153 ± 0.009
Prostate	3.238 ± 0.370	0.028 ± 0.003	0.008 ± 0.001	0.000 ± 0.000
Skin	7.579 ± 0.844	0.067 ± 0.007	0.018 ± 0.001	3.255 ± 0.260
Spleen	7.808 ± 0.804	0.069 ± 0.008	0.019 ± 0.002	0.039 ± 0.002
Testes	6.805 ± 0.379	0.060 ± 0.004	0.016 ± 0.001	0.000 ± 0.000
Thyroid	1571.119 ± 330.362	13.693 ± 2.508	3.741 ± 0.717	0.281 ± 0.054
Urinary Bladder	4.266 ± 0.304	0.038 ± 0.003	0.010 ± 0.001	0.000 ± 0.000

NM-412 14 Day (n=4)	dpm/mg	% dose/gm ± SEM	% kg-does/gm ± SEM	% dose/organ ± SEM
Adrenal	16.812 ± 1.826	0.149 ± 0.011	0.041 ± 0.002	0.010 ± 0.000
Blood	0.646 ± 0.085	0.006 ± 0.001	0.002 ± 0.000	0.080 ± 0.012
Bone Marrow	4.763 ± 0.802	0.042 ± 0.005	0.011 ± 0.001	0.040 ± 0.003
Duodenum	1.787 ± 0.288	0.016 ± 0.002	0.004 ± 0.000	0.083 ± 0.006
Eye	0.869 ± 0.107	0.008 ± 0.001	0.002 ± 0.000	0.003 ± 0.000
Fat	15.230 ± 1.599	0.135 ± 0.007	0.037 ± 0.003	2.651 ± 0.184
Heart	0.976 ± 0.059	0.009 ± 0.000	0.002 ± 0.000	0.007 ± 0.000
Kidney	2.471 ± 0.443	0.022 ± 0.003	0.006 ± 0.001	0.045 ± 0.005
Liver	3.116 ± 0.192	0.028 ± 0.002	0.008 ± 0.001	0.295 ± 0.012
Lung	2.406 ± 0.102	0.021 ± 0.000	0.006 ± 0.000	0.033 ± 0.001
Muscle	0.717 ± 0.188	0.007 ± 0.002	0.002 ± 0.001	0.823 ± 0.241
Plasma	0.677 ± 0.069	0.006 ± 0.000	0.002 ± 0.000	0.045 ± 0.004
Prostate	1.156 ± 0.223	0.010 ± 0.001	0.003 ± 0.000	0.000 ± 0.000
Skin	4.268 ± 0.573	0.038 ± 0.004	0.011 ± 0.002	1.911 ± 0.284
Spleen	4.870 ± 0.334	0.044 ± 0.004	0.012 ± 0.001	0.027 ± 0.002
Testes	6.048 ± 0.353	0.054 ± 0.002	0.015 ± 0.000	0.000 ± 0.000
Thyroid	787.892 ± 100.612	6.988 ± 0.705	1.935 ± 0.199	0.145 ± 0.015
Urinary Bladder	1.439 ± 0.156	0.013 ± 0.002	0.004 ± 0.000	0.000 ± 0.000

TABLE 2. PREDICTED DOSIMETRY TO MIRD ADULT PHANTOM OF ^{131}I-LABELED PHOSPHOLIPID ETHER ANALOGS BASED UPON RAT BIODISTRIBUTION DATA.

TARGET ORGAN	NM-324 rad/mCi	NM-404 rad/mCi	NM-412 rad/mCi
Adrenals	0.646	2.270	1.650
Brain	0.014	0.057	0.018
Breasts	0.069	0.146	0.048
Gallbladder	0.466	0.367	0.145
LLI Wall	0.239	0.259	0.078
Small Intestine	4.070	0.243	0.078
Stomach	0.197	0.265	0.088
ULI Wall	0.507	0.245	0.081
Heart Wall	0.401	1.100	0.289
Kidneys	4.200	1.850	0.701
Liver	2.360	1.260	0.689
Lungs	0.838	2.080	0.697
Muscle	0.229	0.887	0.258
Pancreas	0.281	0.360	0.129
Red Marrow	0.153	0.889	0.335
Bone Surfaces	0.121	0.613	0.222
Skin	0.063	0.137	0.042
Spleen	0.826	1.520	0.696
Testes	0.063	1.430	0.358
Thymus	0.099	0.267	0.082
Thyroid	0.069	3.040	0.068
Urinary Bladder	0.134	0.254	0.074
Total Body	0.318	0.557	0.178

Residence Times: (source organs used)

	NM-324	NM-404	NM-412
Adrenals	0.013	0.073	0.058
Small Intestine	70510		
Heart Wall	0.171	0.575	0.135
Kidneys	2.570	1.020	0.391
Liver	7.940	3.780	2.230
Lungs	1.610	4.170	1.400
Muscle	13.10	45.00	12.80
Red Marrow		3.430	1.360
Spleen	0.252	0.490	0.239
Testes		0.110	0.027
Thyroid		0.135	

TABLE 3. BIODISTRIBUTION OF ^{125}I-NM-412 IN MALE SCID MICE BEARING PC-3 XENOGRAFTS EXPRESSED AS % ADMINISTERED DOSE/GM (MEAN, N=4).

	DAY 1	DAY 3	DAY 5	DAY 8	DAY 14
ADRENAL	6.369	3.028	1.450	1.113	0.700
BLOOD	1.842	0.707	0.371	0.347	0.050
DUODENUM	2.999	1.369	0.615	0.392	0.138
FAT	0.997	1.240	0.422	0.398	0.451
HEART	1.783	0.667	0.326	0.238	0.109
KIDNEY	3.603	1.539	0.591	0.380	0.088
LIVER	4.285	1.438	0.787	0.866	0.279
LUNG	6.032	2.812	1.178	0.674	0.197
MUSCLE	0.527	0.307	0.156	0.114	0.060
PLASMA	8.507	1.253	0.368	0.350	0.070
PROSTATE	1.695	1.212	0.633	0.320	0.130
SPLEEN	4.269	1.686	0.587	0.698	0.175
THYROID	1.933	1.219	0.435	0.300	0.221
TUMOR	2.163	3.230	2.848	2.640	1.839

TUMOR TO NON-TARGET RATIOS OF NM-412 IN MALE SCID MICE BEARING PC-3 XENOGRAFTS.

NON-TARGET TISSUE	DAY 1	DAY 3	DAY 5	DAY 8	DAY 14
ADRENAL	0.34	1.07	1.96	2.37	2.63
BLOOD	1.20	4.57	7.68	7.61	36.84
DUODENUM	0.72	2.36	4.63	6.73	13.32
FAT	2.17	2.60	6.75	6.63	4.08
HEART	1.12	4.84	8.87	11.09	16.91
KIDNEY	0.60	2.10	4.82	6.94	20.96
LIVER	0.50	2.25	3.62	3.05	6.59
LUNG	0.36	2.16	2.42	3.91	9.34
MUSCLE	4.11	10.52	18.27	23.12	30.64
PLASMA	0.24	2.58	7.74	7.55	26.12
PROSTATE	1.28	2.67	4.50	8.26	14.16
SPLEEN	0.51	1.92	4.85	3.78	10.49
THYROID	1.12	2.65	6.55	8.81	8.32

In order to conduct the clinical evaluation of NM-404 in patients with prostate cancer, a number of internal and external applications have had to be submitted. The requirements for such studies have changed substantially and become more rigorous in recent years. *These changes were not anticipated when our grant was originally submitted.* Thus applications were required for the Clinical Research Center of the University of Michigan Hospital, the University's Institutional Review Board for Human Subject Research and the Radioactive Drug Research Committee. Title pages for each of these applications and the responses from the various committees are included in the Appendices 2-4. Needless to say, the completion of these applications as well as the delays encountered in their response has been very time consuming. Moreover, a reinterpretation of the FDA requirements made it necessary to file an Investigational New Drug Application (IND). This application totaling 230 pages was submitted to the FDA on May 29, 2001. The face page of the application and the letter of receipt from the FDA are shown in Appendix 5.

Upon approval of the IND by the FDA, we will formulate and sterilize doses of unlabeled NM-404 for administration to normal males at a dose of five times that to be administered to cancer patients in order to assure the lack of any toxic manifestations associated with the product. Once this is concluded, studies will begin in prostate cancer patients with iodine-131 labeled NM-404 at a maximum dose of 2 mCi per patient. We are anxiously looking forward to receiving the go ahead to conduct these critical studies.

The reviewers of this project are requested to see the appended (Appendix 6) material citing presentation of our studies at various National and International meetings. Our paper presented in September 1999 has now been published as a chapter in a book entitled "Isotope Production and Applications in the 21st Century" edited by Dr. Nigel R. Stevenson. The table in that publication illustrates the strikingly high percentage of the injected dose residing in the prostate tumor as opposed to other tissues. These results coupled with the excellent dosimetry presented by Dr. K. R. Zasadny at the Nuclear Medicine Society annual meeting indicate that NM-404 is a very promising agent for our initial human trials. In addition, Dr. Counsell was invited to give a plenary lecture on our studies with radioiodinated phospholipid ether analogs and their potential for the diagnosis and treatment of prostate cancer at the Fourth International Symposium on Radiohalogens held at Whistler, British Columbia in September, 2000.

7. KEY RESEARCH ACCOMPLISHMENTS:

- Development of an improved method for the radioiodination of NM-404 and NM-412, which leads to products with substantially increased specific activity.
- Completion of biodistribution studies with NM-412 in normal male Sprague-Dawley rats required for dosimetric calculations (Table 1).
- Completion of dosimetry calculations using MIRDOSE 3.1 to determine the appropriate dose of NM-412 for administration to prostate cancer patients (Table 2).
- Successful imaging of prostate cancer with NM-412 in male SCID mice bearing human PC-3 tumor xenografts (see images in Appendix 1).
- Receipt of positive response from The University of Michigan General Clinical Research Center for conduct of patient studies in the Center facilities. (see letter in Appendix 2).
- Submission and positive response to our application to the University's Institutional Review Board (IRB-MED, IRB # 2000-420, see correspondence in Appendix 3).
- Submission of our application (00-123) to the Radioactive Drug Research Committee and the Subcommittee on the Human Use of Radioisotopes (RDRC/SHUR) for approval to conduct a preliminary pharmacokinetic appraisal of ^{131}I-NM-404 in ten (10) prostate cancer patients and the Committee's response (see Appendix 4).
- Compilation and submission of an Investigational New Drug (IND # 62,703)) application to the Food and Drug Administration (FDA) for the study of NM-404 in human subjects as required by 21 CFR 312 and 21 CFR 361.1 (see Appendix 5).

8. REPORTABLE OUTCOMES:

Manuscripts, Abstracts and Presentations:

Zasadny KR, Longino MA, Fisher SJ, Counsell RE and Wahl RL. Predicted Dosimetry for 131-I NM-404, A Phospholipid Ether Agent for Tumor Imaging and Possible Therapy. *J Nucl Med* 40:39P, 1999.

Counsell RE, Longino MA, Pinchuk AN, Skinner RWS, Fisher SJ, Van Dort ME, Pienta KF and Wahl RL. Synthesis and Evaluation of a Radioiodinated Phospholipid Ether Analog (NM-404) for Diagnostic Imaging of Prostate Cancer. *Isotope Production and Applications in the 21st Century*, NR Stevenson, ed., World Scientific, Singapore, pp163-166, 2000.

Counsell RE, Longino MA, Pinchuk AN, Van Dort ME, Fisher SJ, Skinner RWS, Zasadny KR and Wahl RL. Radioiodinated Phospholipid Ethers and Analogs as Tumor Imaging Agents. Fourth International Symposium on Radiohalogens, Whistler, B.C., Canada, September 9-13, 2000.

9. CONCLUSION:

Progress towards our stated goals was dealt a setback when Dr. Richard Wahl left the University to become Professor and Chairman of the Division of Nuclear Medicine at Johns Hopkins Medical School. Dr. Wahl was the P.I. for the project and was specifically responsible for the clinical phases. Despite completing all of the preclinical studies for NM-404 in the first year, progress towards initiating the clinical studies was impeded. Upon Dr. Wahl's departure, Dr. Counsell became overall Principal Investigator in November 2000 and Dr. Milton Gross assumed direction of the clinical studies. This delay necessitated the request for a one year no cost extension and its subsequent approval. Progress, since our last report in July 2000, has included completion of all preclinical studies for our second agent, NM-412, and pursuit of the clinical studies for NM-404. To achieve the latter, all necessary applications for the clinical study have been completed and submitted. This has included applications to the Clinical Research Center, the Institutional Review Board for Human Subject Research (IRB-MED) and the Radioactive Drug Research Committee. In addition, application for an IND for NM-404 was submitted to the FDA in May 2001. We anticipate receiving approval for the clinical studies momentarily.

10. REFERENCES:

Zasadny KR, Longino MA, Fisher SJ, Counsell RE and Wahl RL. Predicted Dosimetry for 131-I NM-404, A Phospholipid Ether Agent for Tumor Imaging and Possible Therapy. *J Nucl Med* 40:39P, 1999.

Counsell RE, Longino MA, Pinchuk AN, Skinner RWS, Fisher SJ, Van Dort ME, Pienta KF and Wahl RL. Synthesis and Evaluation of a Radioiodinated Phospholipid Ether Analog (NM-404) for Diagnostic Imaging of Prostate Cancer. *Isotope Production and Applications in the 21st Century*, NR Stevenson, ed., World Scientific, Singapore, pp163-166, 2000.

Counsell RE, Longino MA, Pinchuk AN, Van Dort ME, Fisher SJ, Skinner RWS, Zasadny KR and Wahl RL. Radioiodinated Phospholipid Ethers and Analogs as Tumor Imaging Agents. Fourth International Symposium on Radiohalogens, Whistler, B.C., Canada, September 9-13, 2000.

11. APPENDICES:

APPENDIX 1: γ-CAMERA IMAGES OF PC-3 SCID MICE INJECTED WITH NM-412

APPENDIX 2: GCRC APPLICATION COVER AND LETTER

APPENDIX 3: IRB-MED APPLICATION COVER AND LETTER

APPENDIX 4 CONSENT FORMS

APPENDIX 5: RDRC APPLICATION COVER AND LETTER

APPENDIX 6: IND APPLICATION COVER SHEET AND RECEIPT LETTER

APPENDIX 7: PUBLICATIONS

APPENDIX 1: γ-CAMERA IMAGES OF PC-3 TUMORS IN SCID MICE INJECTED WITH NM-412

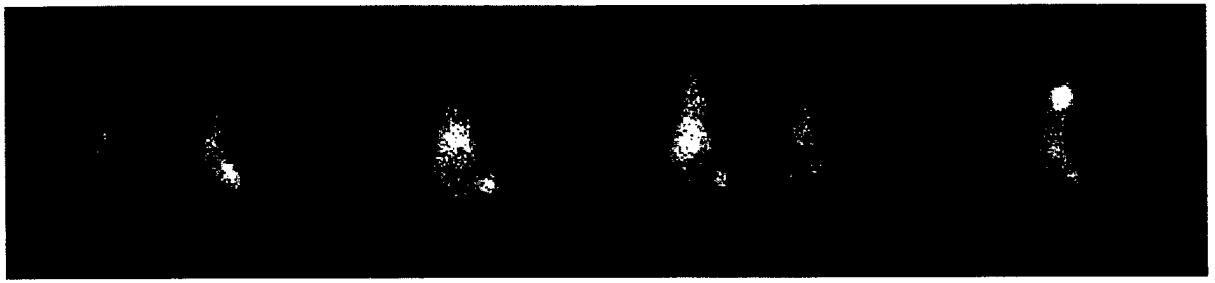
6 MICE

DAYS POST INJECTION

DAY 1

DAY 3

1 2 3 4



1 2 3 4

DAY 5

1 2 3 4

1 2 3 4 5 6

NM 412

PC-3 TUMOR IN SCID MICE

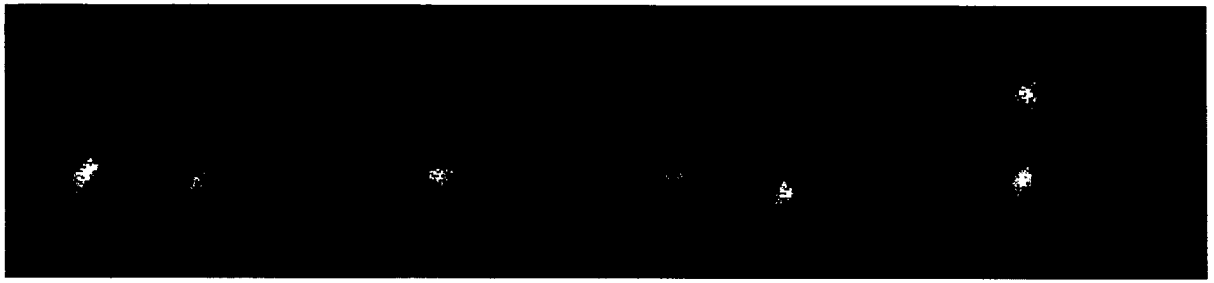
Poor injection mouse #6

38.5 microCi/mouse

6 MICE

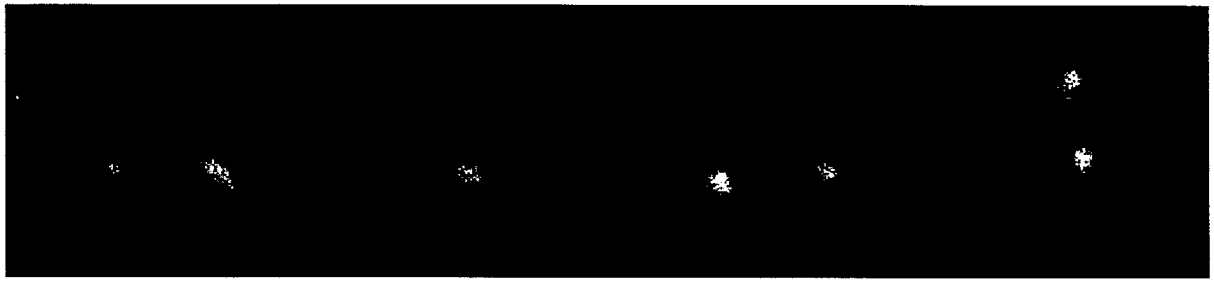
DAYS POST INJECTION

DAY 11



1 2 3 4

DAY 15



1 2 3 4

DAY 26

1 2 3 4

1 2 3 4 5 6

NM 412

PC-3 TUMOR IN SCID MICE

NOTE: ORIENTATION NEEDS TO BE CORRECTED DAY 11

38.5 microCi/mouse

APPENDIX 2: GCRC APPLICATION COVER AND LETTER

The University of Michigan Medical Center
General Clinical Research Center (GCRC)
A7119 University Hospital Box 0108
(734) 936-8080 telephone
(734) 936-4024 fax
http://www.med.umich.edu/gcrc/

NEW APPLICATION
Version 11/1/99

GCRC NARRATIVE

This file is a regular word document (not a checkbox form like Survey.rtf).

Principal Investigator's Name: Milton D. Gross, M.D.

Email address: mdgross@umich.edu

Title of research project:
Clinical evaluation of novel agents for noninvasive imaging of prostate cancer. Subpharmacological and tracer imaging studies using a phospholipid ether analog, NM-404.
Part A: Normal Subjects
Part B: Patients with prostate Cancer

A. Layperson's abstract (250-400 words)

(NIH has asked for this abstract for inclusion in their databases / reports to Congress / general public. Informed Consent document may be a good source. Layperson's abstract should describe background information including why study is being performed, what drugs or other interventions are being studied, populations being targeted, how participants are assigned to a treatment (design), and the outcomes (primary & secondary) being examined for change, e.g. tumor size, weight gain, quality of life, etc.)

We wish to evaluate a new radiopharmaceutical called I-131 NM-404 in patients with prostate cancer to determine whether the compound will accumulate in areas of known cancer following intravenous injection. We will also determine how much of the compound goes to the cancer as opposed to normal tissues, whether there are any adverse effects, and how rapidly the radiopharmaceutical clears from a patient's body. These data will help us predict whether this agent will likely have either diagnostic or therapeutic utility in patients with prostate cancer. All patients recruited for this study will be males (only males get prostate cancer), 21 years and older, of any race.

NM-404 is a phospholipid ether analog (PLE) compound that has been manufactured in our laboratory to target human cancers. The radiolabeled agent has been shown in animals to accumulate into areas of prostate cancer. The agent is investigational and not approved by the Food and Drug Administration (FDA) for commercial use; however, an IND application will be submitted to permit its use in this research study under the Radioactive Drug Research Committee (RDRC) mechanism.

In order to validate the radiolabeled NM-404 for administration to humans with prostate cancer under the RDRC approval, the stable, non-radioactive agent needs to be administered to five normal male volunteers

General Clinical
Research Center
University of Michigan

August 31, 2000

Richard Wahl, M.D.
Professor, Internal Medicine and
Radiology
B1G412 UH
Box 0028

RE: Protocol #1710 – "Clinical Evaluation of Novel Agents for Non-Invasive Imaging of Prostate Cancer. Subpharmacological and Tracer Imaging Studies Using a Phospholipid Ether Analog, NM-404. Part A: Normal Subjects, Part B: Patients with Prostate Cancer." "

Dear Dr. Wahl : Rich

The Advisory Committee of the General Clinical Research Center met on Tuesday, August 29, 2000, to discuss your above cited protocol. The Committee approved the protocol, but had a number of questions to which you should reply in a letter to the Director. One comment was why is there no Oncologist as a Co-Investigator on this study? Why is the sampling for the PSA so frequent? And, why is the laboratory testing so intense? There was also no listing of the toxicities or potential toxicities of the agent in the protocol per the committee reviewer. The Committee is under the assumption that you will shortly be replaced as the P.I. of this protocol by Drs. Gross and Shapiro. Please clarify that transfer in writing. Hopefully, you will be able to answer the questions raised by the external reviewer and by this letter and respond to the Director in order to initiate your protocol in a timely fashion. The Committee approved your submission contingent upon such a letter being generated.

The staff of the GCRC looks forward to assisting you in anyway possible with this interesting protocol.

Best regards,

John Wiley, M.D.
Program Director GCRC

cc: Raymond Counsell, Ph.D.
James Montie, M.D.

A7119 University Hospital, Box 0108
1500 East Medical Center Drive
Ann Arbor, MI 48109-0108

Phone: (734) 936-8080
Fax: (734) 936-4024
http://www.med.umich.edu/gcrc/

To: John Wiley, M.D.

From: Richard Wahl, M.D., Nuclear Medicine

Re: CRC Protocol #1710, Clinical Evaluation of Novel Agents for Non Invasive Imaging of Prostate Cancer. Subpharmacological and Tracer Imaging Studies Using a Phospholipid Ether Analog, NM-404. Part A: Normal Subjects, Part B: Patients with Prostate Cancer"

Date: 10/24/00

Thank you for the thoughtful yet positive review of the above mentioned GCRC proposal. The questions raised are appropriate. Specific responses are:

Why is there no oncologist as there is not a multidisciplinary prostate cancer clinic? Dr. Montie advises me that recruitment should not be difficult as he meets with oncologists on a regular basis in the prostate cancer clinic (i.e. that multiple disciplines participate). He felt the comment was thus not relevant. Obviously, if recruitment is problematic, an oncologist will be sought out. The frequency of PSA testing and intensity of lab testing were raised as issues. The agent is a new one and we are conservatively testing for toxicity, though none is expected. We believe this is well addressed in the IRB documents and consent form for the human studies. The PSA levels are assessed on the remote chance that therapeutic effect would be seen. This is very very unlikely at what we believe to be subpharmacological doses, however pharmacological doses of PLE compounds have been shown to have anti-neoplastic activity levels and this is the reason for the testing. We believe it reasonable, though of probable low yield and request it remain in the protocol.

Finally, today is my last day at the University of Michigan. Dr.Milton Gross, Professor of Radiology and Internal Medicine, has agreed to take over this project as PI. The appropriate changes in the IRB and RDRC approvals are taking place. I will remain a co-investigator, responsible for analysis of the dosimetry data using our established methods.

Thanks to you and the GCRC for the help you have given me on this project and through the past 17 years I have been at the University of Michigan.

For your information, my phone number at Johns Hopkins is 410-614-3764 and my e mail is rwahl@jhmi.edu if questions remain.

Xc: Milton Gross, Raymond Counsell, James Montie, Denise Regan

APPENDIX 3: IRB-MED APPLICATION COVER AND LETTER

The University of Michigan Medical School
Institutional Review Board for Human Subject Research (IRBMED)

2423 Med Sci I/Box 0605

(734) 763-4768 - phone

(734) 763-9603 - fax

http://www.med.umich.edu/irbmed

REQUEST FOR REVIEW OF A NEW RESEARCH PROJECT

(Version May 1999)

BEGINNING OF SECTIONS FOR GENERAL INFORMATION ON THE NEW RESEARCH PROJECT

1. PROJECT IDENTIFICATION

Do not delete this section. Completion of this section is required for all types of projects.

(Please note: In the near future, this section will be completed on-line at the IRBMED Internet Web site. Information will be transferred electronically to the IRBMED electronic database. A printed copy to be produced by the Investigator at that time will be part of this application.)

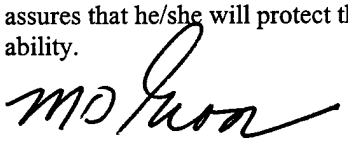
1.1 Principal Investigator's last name, followed by first name:

Only one Principal Investigator may be identified.

Gross, Milton

1.2 Principal Investigator's signature:

Signature of the PI is required on the printed copy of this application document. By signing, the PI assures that he/she will protect the rights and welfare of human research subjects to the best of his/her ability.



1.3 IRBMED Archive Number:

Leave blank; for office use only.

1.4 Title of research project:

Clinical evaluation of novel agents for noninvasive imaging of prostate cancer.
Subpharmacological and tracer imaging studies using a phospholipid ether analog, NM-404.

Part A: Normal Subjects

Part B: Patients with prostate Cancer

1.5 Name of extramural company or agency sponsoring the research:

Enter "NA", if there is no extramural support. A Sponsor outside of the University of Michigan.

U.S. Army Medical Research and Materiel Command (USAMRMC)

1.6 Extramural research sponsor's identifier code:

Enter "NA", if not applicable, or not necessary.

To: Milton Gross

From: Mindy Schmidt

Subject: IRB# 2000-420

CC: Denise Regan

Dr. Gross:

The approval of your project entitled "Clinical evaluation of novel agents for noninvasive imaging of prostate cancer. Subpharmacological and tracer imaging studies using a phospholipid ether analog, NM-404. (Part A: Normal Subjects, Part B: Patients with prostate cancer)" is still pending. SHUR approval must be obtained prior to IRBMED approval being granted. The IRBMED Office has an update from SHUR on file regarding this project's SHUR approval being tabled (letter dated 12/04/00). Please fax a copy of SHUR approval as soon as it is obtained.

Sincerely,

Mindy Schmidt
Institutional Review Board (IRBMED)
University of Michigan
2423 Med Sci I / Box 0605
1301 Catherine Street
Ann Arbor, MI 48109-0605
(734) 615-4835 phone
(734) 763-9603 fax
mindysch@med.umich.edu
http://www.med.umich.edu/irbmed

APPENDIX 4 CONSENT FORMS

The University of Michigan Health System (UMHS), Ann Arbor, Michigan

INFORMED CONSENT OF A SUBJECT PARTICIPATING IN A RESEARCH STUDY

(Version April 1998)

1. GENERAL INFORMATION

The Institutional Review Board for Human Subject Research at the University of Michigan Medical School (IRBMED) reviewed this research project from the standpoint of the protection of human research subjects. The IRBMED found the project to be in compliance with the regulations of the United States Government and of the University of Michigan.

1.1 This version of the consent document was prepared on:

2000/11/27

1.2 This version of the consent document was approved by the IRBMED on:

2000/

1.3 This project's approval by the IRBMED will expire on:

2000/

1.4 The archive number assigned by the IRBMED to this project is:

2000-420

1.5 Names of the investigators responsible for this project:

Milton D. Gross, M.D.
Professor of Internal Medicine

Raymond Counsell, Ph.D.
Professor of Pharmacology

James Montie, M.D.
Professor of Urologic Oncology

Richard L Wahl, M.D.
Professor of Internal Medicine and Radiology

1.6 The title of this research project is:

Clinical evaluation of novel agents for noninvasive imaging of prostate cancer.
Subpharmacological and tracer imaging studies using a phospholipid ether analog, NM-404.

Part B: Patients with Prostate Cancer

1.7 The protocol number assigned to this study is:

U.S. Dept. of Defense grant DAMD 17-98-1-8528
Grant Award: PC 970288

1.8 This research is funded by:

U.S. Army Medical Research and Materiel Command (USAMRMC)

2. INFORMATION ON THE RESEARCH STUDY

2.1 What is the purpose of this research study?

We wish to evaluate a new radiopharmaceutical called I-131 NM-404 in patients with prostate cancer. We want to see how much of the compound goes to the cancer cells in your body and how much to normal tissues, whether there are any adverse effects, and how rapidly the agent clears from your body. The data will help us predict whether this agent will likely have either diagnostic or therapeutic utility in patients with prostate cancer.

The NM-404 is a phospholipid ether analog (PLE) agent that has been manufactured in our laboratory to target human cancers. The agent is investigational and not approved by the Food and Drug Administration (FDA) for commercial use; however, the FDA has allowed its use in this research study under the Radioactive Drug Research Committee (RDRC) mechanism. A preliminary study has been completed using NM-404 in healthy volunteers at a dose 10x the labeled dose, with no adverse reactions. In animal studies, the agent was given at over 6000 times the projected human dose without adverse effects.

The NM-404 has been "radiolabeled" with a radioactive substance called I-131 that can be tracked in your body by nuclear medicine cameras. The radiolabeled compound has been shown in animals to accumulate into areas of known prostate cancer following injection into a vein. The ultimate goal of our investigation is to see how well the I-131 NM-404 agent targets prostate tumors and associated metastases, and to prove that it can be used safely and effectively in humans.

2.2 Who can take part in this study?

We expect to study 10 patients with prostate cancer. If more patients wish to participate than there are openings available, we will randomly select participants from the eligible patient pool.

Participants in the study will be males, 21 or older. They will have had a CT Scan or other objective imaging (bone scan) or physical examination finding within 4 weeks of study entry date showing one or more tumor(s) big enough to find on CT. A positive bone scan is acceptable if there is a new lesion consistent with progressive disease. Biopsy proof of a lesion is preferred but not mandatory for participation in this study. Patients must be in fairly good physical health and have had no chemotherapy or radiation therapy for 6 weeks prior to scan. Extremely ill patients will not be included. Blood work will be obtained prior to the study to meet certain eligibility criteria.

2.3 Why should I consider joining this study as a research subject?

The information obtained from this study will be used scientifically to determine if the agent does indeed detect prostate tumors. Ultimately, we hope to use I-131 NM-404 in even greater doses to achieve a therapeutic effect safely in patients with prostate cancer, if the preliminary studies are promising. Thus, your participation may ultimately provide societal benefit. You will also receive monetary compensation for your time.

2.4 Do I have to become a subject in this study? If I joined the study, can I change my mind and drop out before it ends?

Your becoming a subject in this study is entirely by your own free choice. You may also drop out of the study by your own free will, after having agreed to become a subject. You may refuse to enroll in the study, or drop out of the study at any time. However, payment will only be made upon completion of the study.

2.5 What exactly will be done to me, and what kinds of treatments or procedures will I receive, if I agree to be a research subject in this study?

A CT scan or bone scan must be obtained within 4 weeks of the NM-404 injection. The scan will be for the clinical assessment of the extent of your disease and in most instances will have already been performed for clinical purposes and not specifically for research. It is possible that the timing of the scan may be changed slightly due to your participation in this protocol, however.

You will be admitted to the Clinical Research Center at the University Hospital the day before the injection. Baseline blood and urine tests, an EKG, vital signs (blood pressure, pulse, respiratory rate and temperature), physical examination and a detailed medical history will be obtained. On the day of the infusion, you will have two I.V. lines established, one in each arm. One will be used for blood draws and the other for the injection of the 131-I NM-404. Your vital signs will be checked repeatedly. Multiple blood samples will be obtained throughout the first day.

You will be taken to the nuclear medicine department at 1 hour post-injection to have special pictures taken with a special camera. This camera is very sensitive to the trace levels of radioactivity that have been injected into your body and does not add any radiation exposure to your body. These pictures will be taken at daily intervals during your hospital stay. You will also be asked to collect your urine and feces in labeled containers over the next five days. These 24 hr collections are used to determine how fast the agents disappear from your body through your kidneys and bowels.

At 24 hours following the injection, you will have your baseline blood tests repeated and again at 5 days after injection. These blood tests, along with our observation of you, will be used to determine that there has been no toxicity to you from the I-131 NM-404 injection. The daily blood tests will also be used to help us determine how fast the agent leaves your body. You will be discharged from the hospital following the day 5 (post injection) scan. The images we obtain using the nuclear medicine camera will allow us to determine how much radioactivity reaches your normal tissues and your tumors. Upon discharge from the hospital we may ask that you

return for extra nuclear medicine images and blood draws at approximately 7 to 10 days post-tracer injection, if the day 5 scan shows any remaining radiotracer activity.

2.6 What kinds of harm can I experience in this study, and what will the investigators do to reduce the chances of harm?

Participation in this trial involves several potential risks. These include inconvenience associated with hospitalization or multiple trips to the hospital, blood loss (blood samples are taken over 6 days), boredom, as well as unknown potential adverse effects from the NM-404 compound which in theory could include a severe allergic reaction. The inconvenience associated with multiple imaging times cannot be avoided. As more is learned about the optimal time of imaging, it is possible that the number of imaging times could be reduced, minimizing inconvenience. The amount of blood removed is modest (approximately 15 samples, 2 cc each, plus 6 samples, 8 cc each, for baseline and post injection chemistry analyses). The boredom associated with lying under a gamma camera for 30-60 minutes is unavoidable, but we make available cassette tapes with music as well as occasionally stimulating conversation to break the monotony. The possibility of adverse reaction to NM-404 is considered minimal, in that toxicity studies in rats and rabbits, as well as in normal humans at doses much higher than you will receive have shown no adverse reactions. Thus all pre clinical safety assessments, at doses much larger than planned for you, have demonstrated safety of the NM-404, but we cannot totally rule out the possibility of an adverse or allergic reaction with the very remote possibility of serious injury. All infusions of the radiotracer will be performed in a controlled medical environment and physicians trained in CPR will be available should adverse reactions occur.

While taking part in this study as a subject, as a part of the research, you will be exposed to radiation in the form of gamma and beta radiation. The overall effect of radiation on the human body is measured in terms of Roentgen equivalents in man, or "rem", which is a unit of uniform whole body exposure. Radiation you will be exposed to in this study will amount to less than 5 rem to any tissue, and less than 2.64 rem to your whole body. The effects on your body of this radiation exposure will be added to your overall lifetime radiation risk. Your life-time radiation risk includes the background radiation you are exposed to naturally like everyone else living on this planet, which is on the average 0.3 rem per year; the radiation you will be exposed to in this study is about 9 times the yearly background radiation. In terms of radiation a person may get exposed to during medical care, the amount you will receive in this study will be 264 times the amount of radiation received in routine dental x-rays or chest x-ray, which is 0.01 rem. Federal Government requires that the amount of radiation exposure of radiation workers does not exceed 5 rem per year; the radiation you will be exposed to in this study is one half that amount. Your life-time radiation risk also include any radiation you may have received in the past for diagnosis or treatment, and any such radiation you may be exposed to in the future. Please tell us if you have had any major radiation exposure in the past, particularly in the past two years, such as treatment with x-rays or radioactivity, or diagnostic x-rays, CT-scans or nuclear medicine scans. The risk of harm from radiation exposure of this amount is too small to estimate.

SSKI, a potassium iodide solution, will be given to reduce the radiation dose to the thyroid gland to about 1-2% of what it would be otherwise. The radiation exposure is minimized by choosing a radiation dose that complies with research guidelines. The radiation dose is somewhat higher than that of a bone scan and quite comparable to that of a Gallium scan, a commonly performed

nuclear medicine procedure in patients with cancer.

In addition, participation in multiple studies may be hazardous to you. If you already are participating in another study, please inform us fully. You should not participate in multiple studies, unless you and the investigators agree that your health and the outcome of the study will not be jeopardized.

2.7 What will the investigators do to make sure that the information they will collect on me will not get in wrong hands?

We shall put the information collected about you during the study into a research record. This research record may show your name and registration number, but will be stored in a secure place. We shall keep your research record confidential, to the extent provided by federal, state and local law. We shall not allow anyone to see your record, other than people who have a right to see it. You will not be identified in any reports on this study. The United States Food and Drug Administration, and the sponsor of this research may inspect the research records. Your entire institutional medical record within the University of Michigan Health System, including material not obtained for purposes of this research, may be inspected by the representatives of the U.S. Army Medical Research and Materiel Command (the sponsor of this research) as a part of their responsibility to protect human subjects in research.

It is the policy of the U.S. Army Medical Research and Materiel Command that data sheets are to be completed on all volunteers participating in research for entry into this command's volunteer registry data base. The information to be entered into this confidential database includes your name, address, social security number, study name and dates. The intent of the data base is two-fold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years.

2.8 What kinds of benefit can I expect personally from taking part in this study?

No immediate direct benefit is expected to the participants in this study. While it is conceivable that additional foci of cancer could be identified, this is judged improbable because of the low radioactivity dose we inject. In the longer term, it is possible that targeting sufficient for therapy will be achieved.

2.9 What kinds of benefit to others can come out of this study?

We hope to improve the care and treatment of subsequent patients with prostate cancer. If these initial studies show excellent targeting, these agents may lead to improved diagnostic and therapeutic approach to prostate cancer.

2.10 What will the investigators do, if I get injured in the study?

In the event of a physical injury, which may result from research procedures, the University will provide first aid medical treatment. Additional medical treatment will be provided in accordance with the determination by the University of its responsibility to provide such treatment. However, the University does not provide compensation to a person who is injured while participating as a subject in research.

The United States Department of Defense is funding this research project. Should you be injured as a direct result of participating in this research project, they will provide medical care, at no cost to you, for that injury. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver or release of your legal rights. You should discuss this issue thoroughly with the principal investigator before you enroll in this study.

In case the research participant experiences any discomfort or injury related to this study, the participants should contact:

Milton Gross, M.D.
Division of Nuclear Medicine
University of Michigan Medical Center
Room B1G505C, Box 0028
1500 East Medical Center Drive
Ann Arbor, MI 48109-0028

734-761-7886 or
734-936-6266: pager 2074

2.11 Will I get paid for taking part in this study?

Your participation in this project is voluntary. You will be paid $200 for your participation in this study upon completion.

2.12 Will I or my health insurance company be charged for any of the costs of this study?

The only expenses to you will be for transportation and parking for the scans. No financial burden is anticipated for the health insurance carrier or patient. The CT and bone scans obtained (which may be billed to the insurance) will be part of your general care.

2.13 Once I start in this study as a subject, what do I do if I want to find out more about the study, or to complain about the way I get treated?

If new information is obtained during the course of this research, which may indicate that the risks of harm to subjects have increased significantly, the investigators will let you know, so that you may reconsider your willingness to stay as a subject in the study. To find out more about any aspect of this study, including your rights as a subject, you may contact the persons whose names, addresses and telephone numbers appear above in section 2.10. If you have any questions or concerns about your rights as a research subject, or any grievance, you may also contact the Institutional Review Board For Human Subject Research (IRBMED), 2423 Medical Sciences Building I, 1301 Catherine Street, Ann Arbor, MI 48109-0605; Telephone (734) 763-4768.

2.14 If I decide not to become a subject in this study, what may happen to me, or what other choices do I have if I need treatment?

Alternate procedures to this imaging approach do not exist, however CT and bone scans are not generally sensitive to prostate cancer.

3. DOCUMENTATION OF CONSENT

3.1 Who gets to keep this document, once I sign it?

One copy of this document will be kept together with the investigators' research records on this study. A second copy will be given to the subject to keep. A third copy will be placed in the subject's University of Michigan Health System Medical Record.

3.2 Research subject's statement of consent to participate in this study

I have read the information given above. The investigators personally discussed with me and told me more about the study, and answered my questions. I understand the meaning of this information. I am aware that, like in any research, the investigators cannot always predict what may happen or possibly go wrong. I have been given sufficient time to consider if I should join this study. I hereby consent by my own free choice to take part in the study as a research subject.

3.3 Research subject's identity, and the identity and dated signatures of the subject and/or legal representative of the subject, affirming that consent was given

Subject's Name: _____

Subject's Birth Date: _____

Subject's UMHS Case Number: _____

Adult subject personally giving consent

Consenting Signature of the Subject: _____

Date: _____

3.4 Investigators' confirming statement

I have given this research subject information on the study, which in my opinion is accurate and sufficient for the subject to understand fully the nature, risks and benefits of the study, and the rights of a research subject. There has been no coercion or undue influence. I have witnessed the signing of this document by the subject.

Investigator's Name: _____

Investigator's Signature: _____

Date: _____

The University of Michigan Health System (UMHS), Ann Arbor, Michigan

INFORMED CONSENT OF A SUBJECT PARTICIPATING IN A RESEARCH STUDY

(Version April 1998)

1. GENERAL INFORMATION

The Institutional Review Board for Human Subject Research at the University of Michigan Medical School (IRBMED) reviewed this research project from the standpoint of the protection of human research subjects. The IRBMED found the project to be in compliance with the regulations of the United States Government and of the University of Michigan.

1.1 This version of the consent document was prepared on:

2000/11/27

1.2 This version of the consent document was approved by the IRBMED on:

2000/

1.3 This project's approval by the IRBMED will expire on:

2000/

1.4 The archive number assigned by the IRBMED to this project is:

2000-420

1.5 Names of the investigators responsible for this project:

Milton D. Gross, M.D.
Professor of Internal Medicine

Raymond Counsell, Ph.D.
Professor of Pharmacology

James Montie, M.D.
Professor of Urologic Oncology

Richard L Wahl, M.D.
Professor of Internal Medicine and Radiology

1.6 The title of this research project is:

Clinical evaluation of novel agents for noninvasive imaging of prostate cancer.
Subpharmacological and tracer imaging studies using a phospholipid ether analog, NM-404.

Part A: Normal Subjects

1.7 The protocol number assigned to this study is:

U.S. Dept. of Defense grant DAMD 17-98-1-8528
Grant Award: PC 970288

1.8 This research is funded by:

U.S. Army Medical Research and Materiel Command (USAMRMC)

2. INFORMATION ON THE RESEARCH STUDY

2.1 What is the purpose of this research study?

We wish to evaluate a phospholipid ether analog (PLE) agent called NM-404 for toxic or adverse reactions in normal male volunteers. The agent is investigational and not approved by the Food and Drug Administration (FDA) for commercial use.

This is a preliminary trial to prove that the agent can be safely administered to humans. You will be given a dose approximately ten times the dose that will be given for a subsequent imaging study. This trial follows studies in which rats and rabbits were given >6000x the anticipated human imaging dose with no adverse effects. It also follows studies of a similar PLE compound, NM-324, which also demonstrated no adverse reactions.

The results from this trial will allow us to continue with a subsequent imaging study, using "radiolabeled" NM-404 in patients with prostate cancer. Our overall long term goal is to determine if the radiolabeled agent

can successfully detect and eventually treat prostate cancer.

2.2 Who can take part in this study?

We expect to study 5 normal volunteers with unlabeled NM-404. Participants in the study will be males, 21 or older. Subjects must be in good physical health, with no underlying illnesses that may affect their baseline blood test results. The study will accommodate the first 5 subjects on a first come/ first serve basis.

2.3 Why should I consider joining this study as a research subject?

The information obtained from this study will be used scientifically to determine if the agent may in the future be used as a radiolabeled agent for detection of prostate tumors. Ultimately, if the pilot studies show promise, we hope to use the radiolabeled NM-404 in even greater doses to achieve a therapeutic effect safely in patients with prostate cancer. Thus, your participation may ultimately provide societal benefit. You will also receive monetary compensation for your time.

2.4 Do I have to become a subject in this study? If I joined the study, can I change my mind and drop out before it ends?

Your becoming a subject in this study is entirely by your own free choice. You may also drop out of the study by your own free will, after having agreed to become a subject. You may refuse to enroll in the study, or drop out of the study at any time. However, as a "paid" volunteer, payment will only be made upon completion of the study.

2.5 What exactly will be done to me, and what kinds of treatments or procedures will I receive, if I agree to be a research subject in this study?

After signing this informed consent, you will have your vital signs (blood pressure, pulse, respiratory rate and temperature) taken. A brief physical examination and medical history will also be done. An EKG will be taken. You will be asked for a urine specimen. You will then have an I.V. line established. This will be used for drawing blood and for the injection of the NM-404. Your vital signs will be checked repeatedly, at 30 min., 60 min., 2 hr., 3 hr., and 4 hr.

At 24 and 48 hours following the NM-404 injection, you will have the EKG, blood and urine tests repeated. These tests, along with our observation of you, will be used to determine that there has been no toxicity to you from the NM-404 injection.

2.6 What kinds of harm can I experience in this study, and what will the investigators do to reduce the chances of harm?

Participation in this trial involves several potential risks. These include inconvenience associated with multiple trips to the hospital, blood loss (blood and urine samples are taken over 3 days), boredom, as well as unknown potential adverse effects from the NM-404 compound which in theory could include a severe allergic reaction. The inconvenience associated the follow-up blood work cannot be avoided. The amount of blood removed is modest (approximately 6 samples total). The boredom associated with waiting between times for vital signs is unavoidable, but we make available magazines or television as well as occasionally stimulating conversation to break the monotony. The possibility of adverse reaction to NM-404 is considered minimal, in that toxicity studies in rats and rabbits, at doses much higher than you will receive, have shown no adverse reactions. Prior experience with a similar agent, NM-324, demonstrated no adverse effects in humans. Thus all pre clinical safety assessments, at doses much larger than planned for you, have demonstrated safety of the NM-404, but we cannot totally rule out the possibility of an adverse or allergic reaction with the very remote possibility of serious injury. All infusions of the NM-404 will be performed in a controlled medical environment and physicians trained in CPR will be available should adverse reactions occur.

In addition, participation in multiple studies may be hazardous to you. If you already are participating in another study, please inform us fully. You should not participate in multiple studies, unless you and the investigators agree that your health and the outcome of the study will not be jeopardized.

2.7 What will the investigators do to make sure that the information they will collect on me will not get in wrong hands?

We shall put the information collected about you during the study into a research record. This research record may show your name and registration number, but will be stored in a secure place. We shall keep your research record confidential, to the extent provided by federal, state and local law. We shall not allow anyone to see your record, other than people who have a right to see it. You will not be identified in any reports on this study. The United States Food and Drug Administration, and the sponsor of this research may inspect the research records. Your entire institutional medical record within the University of Michigan Health System, including material not obtained for purposes of this research, may be inspected by the representatives of the U.S. Army Medical Research and Materiel Command (the sponsor of this research) as a part of their responsibility to protect human subjects in research.

It is the policy of the U.S. Army Medical Research and Materiel Command that data sheets are to be completed on all volunteers participating in research for entry into this command's volunteer registry data base. The information to be entered into this confidential database includes your name, address, social security number, study name and dates. The intent of the data base is two-fold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years.

2.8 What kinds of benefit can I expect personally from taking part in this study?

No immediate direct benefit is expected to the participants in this study.

2.9 What kinds of benefit to others can come out of this study?

We hope to improve the care and treatment of patients with prostate cancer. These initial studies must be done in order to prove that the unlabeled agent is safe to use. We hope to later show that this agent, labeled with a radioactive tracer, may lead to an improved diagnostic and possibly therapeutic approach to prostate cancer.

2.10 What will the investigators do, if I get injured in the study?

In the event of a physical injury, which may result from research procedures, the University will provide first-aid medical treatment. Additional medical treatment will be provided in accordance with the determination by the University of its responsibility to provide such treatment. However, the University does not provide compensation to a person who is injured while participating as a subject in research.

The United States Department of Defense is funding this research project. Should you be injured as a direct result of participating in this research project, they will provide medical care, at no cost to you, for that injury. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver or release of your legal rights. You should

discuss this issue thoroughly with the principal investigator before you enroll in this study.

In case the research participant experiences any discomfort or injury related to this study, the participants should contact:

Milton Gross, M.D.
Division of Nuclear Medicine
University of Michigan Medical Center
Room B1G412, Box 0028
1500 East Medical Center Drive
Ann Arbor, MI 48109-0028

734-761-7886 or
734-936-6266: pager 2074

2.11 Will I get paid for taking part in this study?

Your participation in this project is voluntary. You will be paid $200 for your participation in this study upon completion of the 48 hour follow-up laboratory work.

2.12 Will I or my health insurance company be charged for any of the costs of this study?

No.

2.13 Once I start in this study as a subject, what do I do if I want to find out more about the study, or to complain about the way I get treated?

If new information is obtained during the course of this research, which may indicate that the risks of harm to subjects have increased significantly, the investigators will let you know, so that you may reconsider your willingness to stay as a subject in the study. To find out more about any aspect of this study, including your rights as a subject, you may contact the persons whose names, addresses and telephone numbers appear above in section 2.10. If you have any questions or concerns about your rights as a research subject, or any grievance, you may also contact the Institutional Review Board For Human Subject Research (IRBMED), 2423 Medical Sciences Building I, 1301 Catherine Street, Ann Arbor, MI 48109-0605; Telephone (734) 763-4768.

2.14 If I decide not to become a subject in this study, what may happen to me, or what other choices do I have if I need treatment?

Only healthy normal volunteers are to be studied in this preliminary trial.

2 DOCUMENTATION OF CONSENT

3.1 Who gets to keep this document, once I sign it?

One copy of this document will be kept together with the investigators' research records on this study. A second copy will be given to the subject to keep. A third copy will be placed in the subject's University of Michigan Health System Medical Record.

3.2 Research subject's statement of consent to participate in this study

I have read the information given above. The investigators personally discussed with me and told me more about the study, and answered my questions. I understand the meaning of this information. I am aware that, like in any research, the investigators cannot always predict what may happen or possibly go wrong. I have been given sufficient time to consider if I should join this study. I hereby consent by my own free choice to take part in the study as a research subject.

3.3 Research subject's identity, and the identity and dated signatures of the subject and/or legal representative of the subject, affirming that consent was given

Subject's Name: _____

Subject's Birth Date: _____

Subject's UMHS Case Number: _____

Adult subject personally giving consent

Consenting Signature of the Subject: _____

Date: _____

3.4 Investigators' confirming statement

I have given this research subject information on the study, which in my opinion is accurate and sufficient for the subject to understand fully the nature, risks and benefits of the study, and the rights of a research subject. There has been no coercion or undue influence. I have witnessed the signing of this document by the subject.

Investigator's Name: _____

Investigator's Signature: _____

Date: _____

APPENDIX 5: RDRC APPLICATION COVER AND LETTER

100-125 REVISED

Form RSS-102
Revision 9/95

University Of Michigan
Occupational Safety & Environmental Health
Radiation Safety Service

APPLICATION FOR APPROVAL OF HUMAN USE OF RADIOISOTOPES FOR RESEARCH

1.0 NAME, DEPARTMENT, UNIVERSITY ADDRESS, AND TELEPHONE NUMBER OF PRINCIPAL INVESTIGATOR.

Milton Gross, M.D.
Division of Nuclear Medicine
B1G412 University Hospital, Box 0028
734.761.7886

2.0 NAME, DEPARTMENT, AND UNIVERSITY ADDRESS OF CO-INVESTIGATORS.

Raymond Counsell, Ph.D.
Department of Pharmacology
1220C MSRB III, Box 0632
i. 734-764-0251

James Montie, M.D.
Department of Urology- Surgery
2916 Taubman Center, Box 0330

Richard L. Wahl, M.D.
Division of Nuclear Medicine
B1G412 University Hospital, Box 0028
734-936-5384

3.0 SUMMARIZE THE PAST EXPERIENCE AND TRAINING IN THE USE OF RADIOISOTOPES, FOR ALL THE INDIVIDUALS NAMED IN 1.0 AND 2.0 ABOVE.

Dr. Gross is board certified in Nuclear Medicine. He has > 25 years experience in studies with experimental pharmaceuticals in patients including testing new agents for diagnosis and treatment.

Dr. Counsell has >30 years experience in the design, synthesis and evaluation of new radiopharmaceuticals as organ or tumor imaging agents.

Dr. Wahl is board certified in Nuclear Medicine. He has >18 years experience in studies with experimental radiopharmaceuticals in patients including testing new agents for diagnosis and treatment.

Dr. Montie is a specialist in Urologic Oncology and will be useful in identifying patients with prostate cancer for these trials.

4.0 NAME OF STUDY.

Clinical evaluation of novel agents for noninvasive imaging of prostate cancer.
Subpharmacological and tracer imaging studies using a phospholipid ether analog, NM-404.

Part A: Normal Subjects
Part B: Patients with Prostate Cancer

5.0 NAME THE AUTHORIZED USER RESPONSIBLE FOR RADIOISOTOPE ADMINISTRATION, WHO HAS AN RSS-101 AUTHORIZATION TO ORDER THE RADIOISOTOPES INVOLVED IN THIS STUDY. GIVE THE RSS-101 APPROVAL NUMBER.

David E. Kuhl, M.D., RCS-101 Approval # 102R-94-156

University of Michigan
Health System

1500 E. Medical Center Drive
Ann Arbor, Michigan 48109-0028

Department of Radiology
Division of Nuclear Medicine
B1-G412 University Hospital
PHONE (734) 936-5385
FAX (734) 936-8182

April 9, 2001

Dr. Milton Gross
Veterans Affairs Medical Center
Nuclear Medicine Section
2215 Fuller Road
Ann Arbor, MI 48105

Dear Dr. Gross:

On April 4, 2001 the Radioactive Drug Research Committee (RDRC) and Subcommittee on the Human Use of Radioisotopes (SHUR) met to consider your application: 00-123 "Clinical evaluation of novel agents for noninvasive imaging of prostate cancer. Subpharmacological and tracer imaging studies using a phospholipid ether analog, NM-404. Part A: Normal Subjects. Part B: Patients with Prostate Cancer" (Clarifications), Milton Gross, MD.

This was further discussed. Mr. Carey has contacted Dr. Zasadny on the need to account for the total radioactivity and continues to work on this. If possible, the dosimetry to the prostate should also be provided.

The investigator is reminded that the RDRC cannot grant permission for studies of the "cold" material. This must be obtained from the IRB or documentation obtained from the literature or other sources.

I hope you find this satisfactory.

Sincerely,

B. Shapiro, MB, Ch.B., Ph.D.
Chairman Radioactive Drug Research Committee and
Subcommittee on the Human Use of Radioisotopes

jac

APPENDIX 6: IND APPLICATION COVER SHEET AND RECEIPT LETTER

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

INVESTIGATIONAL NEW DRUG APPLICATION (IND)

(TITLE 21, CODE OF FRDERAL REGULATIONS (CFR) PART 312)

Form Approved: OMB No. 0910-0014.
Expiration Date: September 30, 2002
See OMB Statement on Reverse

NOTE: No drug may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40).

1. NAME OF SPONSOR
Dr. Milton D. Gross, M.D.

2. DATE OF SUBMISSION
5/29/01

3. ADDRESS *(Number, Street, City, State and Zip Code)*
University of Michigan, Department of Radiology
B1G 505C, Univ. Hospital, 1500 E. Med. Ctr. Dr., Ann Arbor, MI 48109-0028

4. TELEPHONE NUMBER *(Include Area Code)*
734.761.7886
734.761.5229 Fax

5. NAME(S) OF DRUG *(Include all available names: Trade, Generic, Chemical, Code)*
18-(p-Iodophenyl)-octadecyl phosphocholine, NM-404
Administered in 2% Tween 20-Sterile water

6. IND NUMBER *(If previously assigned)*
62,703

7. INDICATION(S) (Covered by this submission)
Clinical Evaluation of Novel Agents for Noninvasive Imaging of Prostate Cancer. Sub-pharmacological and Tracer-Imaging Studies Using a Phospholipid Ether Analog, NM-404.

8. PHASE(S) OF CLINICAL INVESTIGATION TO BE CONDUCTED:
☒ PHASE 1 ☐ PHASE 2 ☐ PHASE 3 ☐ OTHER _____ *(specify)*

9. LIST NUMBERS OF ALL INVESTIGATIONAL NEW DRUG APPLICATIONS *(21 CFR Part 312)*, NEW DRUG OR ANTIBIOTIC APPLICATIONS *(21 CFR Par 314)*, DRUG MASTER FILES *(21 CFR Part 314.420)*, AND PRODUCT LICENSE APPLICATIONS *(21 CFR Part 601)* REFERRED TO IN THIS APPLICATION.

10. ***IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 000." The next submission (e.g., amendment, report or correspondence) should be numbered "Serial number: 001." Subsequent submissions should be numbered consecutively in the order in which they are submitted.***

SERIAL NUMBER
0 0 0

11. THIS SUBMISSION CONTAINS THE FOLLOWING (Check all that apply)

☒ INITIAL INVESTIGATIONAL NEW DRUG APPLICATION (IND) ☐ RESPONSE TO CLINICAL HOLD

PROTOCOL AMENDMENT(S):
☐ NEW PROTOCOL
☐ CHANGE IN PROTOCOL
☐ NEW INVESTIGATOR

INFORMATION AMENDMENT(S):
☐ CHEMISTRY/ MICROBIOLOGY
☐ PHARMACOLOGY/ TOXICOLOGY
☐ CLINICAL

IND SAFETY REPORT(S):
☐ INITIAL WRITTEN REPORT
☐ FOLLOW-UP TO A WRITTEN RERPORT

☐ RESPONSE TO FDA REQUEST FOR INFORMATION ☐ ANNUAL REPORT ☐ GENERAL CORRESPONDENCE

☐ REQUEST FOR REINSTATEMENT OF IND THAT IS WITHDRAWN, INACTIVATED, TERMINATED OR DISCONTINUED ☐ OTHER _____ *(Specify)*

CHECK ONLY IF APPLICABLE

JUSTIFICATION STATEMENT MUST BE SUBMITTED WITH APPLICATION FOR ANY CHECKED BELOW. REFER TO CITED CFR SECTIION FOR FURTHER INFORMATION

☐ TREATMENT IND 21 CFR 312.35(b) ☐ TREATMENT PROTOCOL 21 CFR 312.35(a) ☐ CHARGE REQUEST/ NOTIFICATION 21 CFR 312.7(d)

FOR FDA USE ONLY

CDR/DBIND/DGD RECEIPT STAMP	DDR RECEIPT STAMP	DIVISION ASSIGNMENT:
		IND NUMBER ASSIGNED:

Created by: PSC Media Arts Branch (301) 443-2454 EF

12. **CONTENTS OF APPLICATION**

This application contains the following items: *(Check all that apply)*

- ☒ 1. Form FDA 1571 *[21 CFR 312.23(a)(1)]*
- ☒ 2. Table of Contents *[21 CFR 312.23(a)(2)]*
- ☒ 3. Introductory statement *[21 CFR 312.23(a)(3)]*
- ☒ 4. General investigational plan *[21 CFR 312.23(a)(3)]*
- ☐ 5. Investigator's brochure *[21 CFR 312.23(a)(5)]*
- ☒ 6. Protocol(s) *[21 CFR 312.23(a)(6)]*
  - ☒ a. Study protocol(s) *[21 CFR 312.23(a)(6)]*
  - ☒ b. Investigator data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  - ☒ c. Facilities data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  - ☒ d. Institutional Review Board data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
- ☒ 7. Chemistry, manufacturing, and control data *[21 CFR 312.23(a)(7)]*
  - ☒ Environmental assessment or claim of exclusion *[21 CFR 312.23(a)(7)(iv)(e)]*
- ☒ 8. Pharmacology and toxicology data *[21 CFR 312.23(a)(8)]*
- ☒ 9. Previous human experience *[21 CFR 312.23(a)(9)]*
- ☒ 10. Additional information *[21 CFR 312.23(a)(10)]*

13. IS ANY PART F THE CLINICAL STUDY TO BE CONDUCTED BY A CONTRACT RESEARCH ORGANIZATION? ☐ YES ☒ NO

IF YES, WILL ANY SPONSOR OBLIGATIONS BE TRANSFERRED TO THE CONTRACT RESEARCH ORGANIZATION? ☐ YES ☐ NO

IF YES, ATTACH A STATEMENT CONTAINING THE NAME AND ADDRESS OF THE CONTRACT RESEARCH ORGANIZATIION, IDENTIFICATION OF THE CLINICAL STUDY, AND A LISTING OF THE OBLIGATIONS TRANSFERRED.

14. NAME AND TITLE OF THE PERSON RESPONSIBLE FOR MONITORING THE CONDUCT AND PROGRESS OF THE CLINICAL INVESTIGATIONS

Dr. Milton D. Gross, M.D., Professor of Radiology and Internal Medicine

15. NAMES AND TITLE(S) OF THE PERSON(S) RESPONSIBLE FOR REVIEW AND EVALUATION OF INFORMATION RELEVANT TO THE SAFETY OF THE DRUG

Dr. Milton D. Gross, M.D., Professor of Radiology and Internal Medicine

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by the FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set forth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

16. NAME OF SPONSOR OR SPONSOR'S AUTHORIZED REPRESENTATIVE	17. SIGNATURE OF SPONSOR OR SPONSOR'S AUTHORIZED REPRESENTATIVE
Dr. Milton D. Gross, M.D., Professor of Radiology and Internal Medicine	MD Gross

18. ADDRESS *(Number, Street, City, State and Zip Code)*	19. TELEPHONE NUMBER *(Include Area Code)*	20. DATE
University of Michigan, Department of Radiology B1G 505C, Univ. Hospital, 1500 E. Med. Ctr. Dr., Ann Arbor, MI 48109-0028	734.761.7886	5/29/01

(**WARNING**: A willfully false statement is a criminal offense. U.S.C. Title 18, Sec. 1001.)

Public reporting burden for this collection of information is estimated to average 100 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Food and Drug Administration
CBER (HFM-99)
1401 Rockville Pike
Rockville, MD 20852-1448

Food and Drug Administration
CDER (HFD-94)
5516 Nicholson Lane
Kensington, MD 20895

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

Please **DO NOT RETURN** this application to this address.

Created by: PSC Media Arts Branch (301) 443-2454 EF

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

IND 62,703

Milton D. Gross, M.D.
University of Michigan
Department of Radiology
B1G 505C - University Hospital
1500 E. Medical Center Dr.
Ann Arbor, MI 48109-0028

Dear Dr. Gross:

We acknowledge receipt of your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act. Please note the following identifying data:

IND Number Assigned:	62,703
Sponsor:	Milton D. Gross, M.D.
Name of Drug:	NM-404
Date of Submission:	May 29, 2001
Date of Receipt:	May 31, 2001

Studies in humans may not be initiated until 30 days after the date of receipt shown above. If, on or before June 30, 2001, we identify deficiencies in the IND that require correction before human studies begin or that require restriction of human studies, we will notify you immediately that (1) clinical studies may not be initiated under this IND ("clinical hold") or that (2) certain restrictions apply to clinical studies under this IND ("partial clinical hold"). In the event of such notification, you must not initiate or you must restrict such studies until you have submitted information to correct the deficiencies, and we have notified you that the information you submitted is satisfactory.

It has not been our policy to object to a sponsor, upon receipt of this acknowledgement letter, either obtaining supplies of the investigational drug or shipping it to investigators listed in the IND.

However, if the drug is shipped to investigators, they should be reminded that studies may not begin under the IND until 30 days after the IND receipt date or later if the IND is placed on clinical hold.

As sponsor of this IND, you are responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the implementing regulations (Title 21 of the Code of Federal Regulations). Those responsibilities include (1) reporting any unexpected fatal or life-threatening adverse experience associated with use of the drug by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]; (2) reporting any adverse experience associated with use of the drug that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]; and (3) submitting annual progress reports [21 CFR 312.33].

Please forward all future communications concerning this IND in triplicate, identified by the above IND number, to the following address:

U.S. Postal Service/Courier/Overnight Mail:
Food and Drug Administration
Center for Drug Evaluation and Research
Division of Medical Imaging and Radiopharmaceutical Drug Products
Attention: Division Document Room, 18B-06
5600 Fishers Lane, HFD-160
Rockville, Maryland 20857

If you have any questions, please call Thuy M. Nguyen, M.P.H., Regulatory Health Project Manager, at (301) 827-7510.

Sincerely,

{See appended electronic signature page}

Kyong Cho, Pharm.D.
Chief, Project Management Staff
Division of Medical Imaging and
Radiopharmaceutical Drug Products
Office of Drug Evaluation III
Center for Drug Evaluation and Research

APPENDIX 7: PUBLICATIONS

Radiopharmaceutical Chemistry Track
Dosimetry: Clinical Dosimetry

2:15 PM-3:45 PM **Session 23** **Room: 403 B**

Moderator: Barry W. Wessels, PhD
Co-Moderator: John L. Humm, PhD

No. 155

PREDICTED DOSIMETRY FOR I-131-NM-404, A PHOSPHOLIPID ETHER AGENT FOR TUMOR IMAGING AND POSSIBLE THERAPY. K. R. Zasadny*, M. A. Longino, S. J. Fisher, R. E. Counsell, R. L. Wahl, The University of Michigan Medical Center, Ann Arbor, MI. (500384)

Objectives: Phospholipid ether agents have the potential to image and possibly deliver therapeutic radiation to a wide variety of human tumors due to their differentially slower metabolism in tumors relative to normal tissues. Previous phospholipid ether agents have successfully targeted a variety of human neoplasms including colon, lung and ovarian cancer. Iodine-labeled NM-404 has successfully targeted tumors in the rat including the Walker256 tumor line. This study focuses on predicted normal organ dosimetry for I-131-labeled NM-404 for humans based on biodistribution studies in the rat. Methods: Tissue distribution studies were carried out after I-125-labeled NM-404 injection in male Sprague-Dawley rats at six time points (3 animals per time point): 1 hr, 6 hr, 24 hr, 72 hr, 7 d and 10 d post injection. Kg*%ID/g uptake in tissues were calculated. Time-activity curves were fit by non-linear least-squares regression using a biexponential model. Extrapolation to human was accomplished by scaling by the total body and organ masses of the MIRD reference adult phantom. Fit time-activity curves were corrected for I-131 decay and integrated to determine dosimetric residence times for the following source organs: adrenals, heart, kidneys, liver, lungs, muscle, marrow, spleen, testes and thyroid (unblocked). The MIRDOSE 3.1 program was used to produce dose estimates. Results: The NM-404 pharmacokinetics show a rapid clearance from the blood followed by a long-lived component. Normal tissues generally show rapid uptake followed by slow clearance. Highest normal organ dose estimates (mGy/MBq) for I-131-labeled NM-404 for the reference adult were seen in thyroid (unblocked) (0.82), followed by adrenals (0.61), lungs (0.56), kidneys (0.50), spleen (0.41), testes (0.39) and liver (0.34). The dose-limiting organ is the testes, with a 3 cGy dose resulting from a 78 MBq administration. Conclusion: Predicted I-131-labeled NM-404 dosimetry results indicate clinically-useful activities for imaging may safely be injected in humans with thyroid blocking. Phase I studies in humans are planned using a 74 MBq (2 mCi) dose.

No. 156

OPTIMIZING COMBINATION THERAPY WITH RADIOLABELED ANTIBODIES AND EXTERNAL BEAM. J. L. Humm*, S. Ruan, S. M. Larson, J. A. O'Donoghue, Memorial Sloan-Kettering Cancer Center, New York, NY. (100338)

Objective: To determine the optimum sequence for combined modality therapy with radiolabeled antibodies and fractionated external beam. **Methods:** The uptake and distribution of I-131 labeled tumor specific A33 monoclonal antibody was determined in SW1222 human colon carcinoma xenografts in nude mice for four study groups (4 animals per group): (1) radiolabeled antibody alone, i.e. pre-radiation therapy controls, (2) antibody administered (day 0) immediately prior to the first of five 2 Gy daily fractions of 320 kVp X-rays, (3) antibody administered after the 5th radiation fraction (day 5), (4) antibody administered five days post irradiation (on day 10). Tumors were excised 5 days post antibody administration. The %injected dose per gram was calculated. Tumors were frozen and sectioned for histology and phosphor imaging autoradiography. The percentage of antigen expressing cells was measured by immunohistochemistry. **Results:** The average tumor uptake relative to control group 1 were 1.47 (group 2), 0.78 (group 3) and 0.21 (group 4) respectively. This illustrates that tumor uptake is increased by almost 50% when the antibody is present in blood at the start of irradiation. 5 days into a fractionated irradiation protocol, antibody uptake was reduced, falling more significantly on day 10. Autoradiographs demonstrated decreased uptake uniformity for groups 3 and 4. Immunohistochemistry showed a reduction in A33 antigen positive cells from 85, 64, 50 to 41% for groups 1-4 respectively. **Conclusions:** Radioimmunotherapy should be administered just prior to the initiation of a course of external beam for maximum tumor uptake and radiolabeled antibody dose. Radiation therapy appears to cause a transient increase in capillary leakage to macromolecules, followed by a reduction at later times possibly the result of capillary damage and occlusion.

No. 157

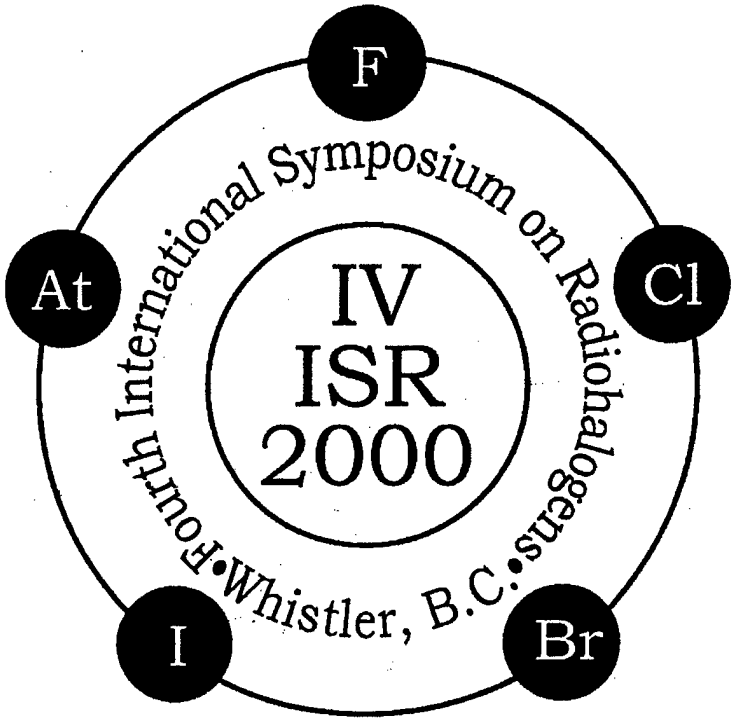
NEW ADDITIONAL MIRD MODEL BASED ADULT PHANTOMS OF DIFFERENT SIZE FOR INTERNAL DOSIMETRY IMPROVEMENT. I. Clairand*, M. Ricard, M. Durigon, M. Di Paola, B. Aubert, Institut Gustave-Roussy, Villejuif, France; Institut Gustave-Roussy and U494 INSERM, Villejuif, France; Hopital Raymond-PoincarZ, Garches, France. (500325)

Objectives: In internal dosimetry, patient morphology is represented by a limited number of models. In the MIRD schema, the adult male phantom is an individual measuring 1.74 m and weighing 70 kg, the adult female is represented by 1.64 m and 58 kg. In order to work with more realistic models, we defined additional MIRD based mathematical anthropomorphic phantoms which represent the physical differences encountered in the adult population. The influence of these morphologic variations on the S-factors was studied. Methods: The analysis of anthropometric data gathered from a legal medicine department (355 men and 329 women of Caucasian type) showed that the mass of most organs is statistically correlated with the height of the body. This led us to develop 3 mathematical male phantoms of 1.60 m, 1.70 m and 1.80 m and 3 female phantoms of 1.50 m, 1.60 m and 1.70 m. These phantoms were built using combinatorial geometry. The S-factors for all the usual target organs were then calculated using a home made Usercode DOSE3D based on the EGS4 Monte Carlo code, when I-131 is uniformly distributed in the stomach and the urinary bladder. Results: An increase in the phantom height by 10 cm leads to a mean S-factor reduction by 20 % when the stomach is the source organ and by 29 % in the case of the urinary bladder. When the phantom height increase is 20 cm, the values are 35 % and 48 %. In some cases, especially when the target organ is far away from the source organ, the differences are 4 fold or more. Conclusion: This work showed the influence of the morphology on the S-factors. The development of new mathematical adult phantoms should contribute to improve dosimetric estimations by taking into account more realistic geometric parameters.

No. 158

ERROR ANALYSIS OF GAMMA CAMERA BASED DOSIMETRY IN RADIOIMMUNOTHERAPY. K. A. Hamacher*, G. Sgouros, Memorial Sloan-Kettering Cancer Center, New York, NY. (100032)

Objectives: The aim of the work presented here was to implement a detailed method to evaluate the error associated with the calculation of the absorbed dose to normal organs in patients undergoing radioimmunotherapy. Methods: The overall uncertainty in absorbed dose is assumed to include errors in (1) estimation of organ activity at multiple time-points from radionuclide imaging and (2) estimation of organ volume. Organ activity quantification is comprised of the following measurements, each of which will have its own uncertainty: attenuation correction, scatter correction, camera calibration, selection of an appropriate background region-of-interest, and selection of a region-of-interest for the organ. Several of these measurements are comprised of a number of independent measurements which themselves are subject to uncertainty. The uncertainty in organ volume quantification will be highly dependent upon the technique used to estimate organ volume with CT or MRI-based measurements being the most accurate and estimation based upon nuclear medicine imaging being less accurate. Error values were assigned to each of the measurements identified above and then propagated to obtain the uncertainty in calculated absorbed dose. Uncertainties were calculated assuming dosimetry was based upon imaging In^{-111}, I^{-131}, or Bi^{-213}. Uncertainty values were determined for volume estimates based upon CT/MRI, SPECT and also estimates based upon organ projections obtained from planar imaging. **Results and Conclusion:** A formalism has been established which provides the uncertainty associated with conventional absorbed dose calculations. This analysis makes it possible to quantitatively identify those elements that contribute the largest uncertainty to absorbed dose estimates, thereby pointing to areas where improvement would be most beneficial.



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Radioiodinated Phospholipid Ethers and Analogs as Tumor Imaging Agents

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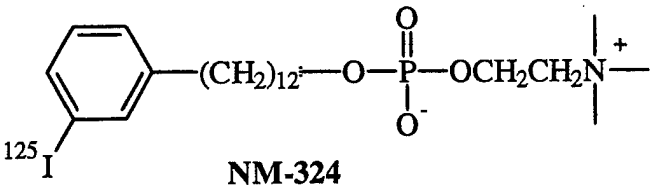
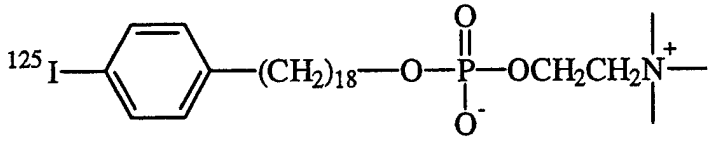
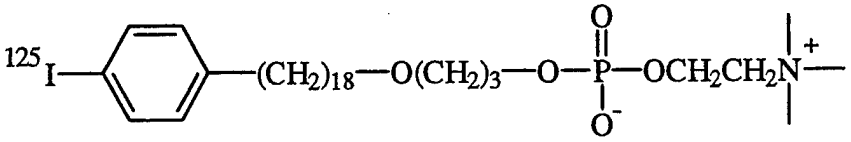
Based upon reports that human tumor tissue contains significantly higher levels of phospholipid ether (PLE) than adjacent normal tissue, our laboratory designed and synthesized a number of radioiodinated PLE analogs as potential tumor imaging agents. Several of these agents showed a striking ability to be taken up and retained by a variety of animal tumors and human tumor xenografts. In an effort to establish the relevance of our animal models to the human situation, one candidate (NM-324) was selected for further preclinical evaluation and subsequently studied in cancer patients. Such studies revealed that NM-324 was capable of imaging tumors in patients, but the high first pass clearance by the liver severely compromised its clinical utility as a diagnostic radioiopharmaceutical. Conversely, this study demonstrated that our animal models were appropriate for the identification of clinical candidates. Therefore, the design of second-generation candidates was focused on those that would possess a longer plasma half-life and/or more rapid metabolic clearance by the liver and other non-target tissues. Two animal models were employed for these studies, namely: SCID mice bearing 1) human lung adenocarcinomas (A549) and 2) human prostate cancer (PC-3). Based upon biodistribution and whole body imaging, two candidates (NM-404 and NM-412) were observed to be superior to NM-324. Moreover, toxicological analysis has shown both NM-404 and NM-412 to have no physiologic or pathologic effects in rats or rabbits at a dose significantly greater than 200 times the anticipated human dose. Phase I trials with both these agents in cancer patents are planned.



NM-324



NM-404



NM-412

SYNTHESIS AND EVALUATION OF A RADIOIODINATED PHOSPHOLIPID ETHER ANALOG (NM-404) FOR DIAGNOSTIC IMAGING OF PROSTATE CANCER

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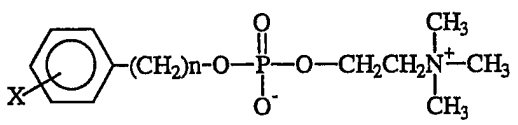
Imaging procedures play a major role in the current management of patients with prostate cancer. Despite advances in many of these procedures, improvements are still needed, especially in the area of Nuclear Medicine. The radioiodinated phospholipid ether analog (PLE) described here represents a new class of radiopharmaceutical, which has provided excellent images of prostate tumors in animal models and is now undergoing preclinical human pharmacokinetic evaluation.

Design and synthesis of radioiodinated PLE was based on the fact that various animal and human tumors contain higher concentrations of ether lipids than surrounding normal tissues. A number of radioiodinated PLE were synthesized and evaluated by γ-camera imaging using rat tumor models as well as nude and SCID mice bearing human tumor xenografts. Of the several agents that displayed promising results, one candidate (NM-324) was selected for further preclinical evaluation and subsequently studied in cancer patients in an effort to ascertain its ability to be retained in human tumors. These studies revealed that NM-324 was capable of imaging tumors in patients, but the high first pass clearance by the liver severely compromised its clinical utility as a diagnostic radiopharmaceutical. Conversely, this study demonstrated that our animal models were appropriate for the identification of clinical candidates.

In an effort to obtain a more suitable clinical candidate, the present study undertook the synthesis and evaluation of additional radioiodinated PLE with a focus on those displaying good tumor avidity and a prolonged plasma half-life relative to the prototype. Biodistribution analysis and γ-camera imaging of Copenhagen rats bearing Dunning R3327 prostate tumors and SCID mice bearing human prostate cancer (PC-3) revealed NM-404 to display a longer plasma half-life, better tumor/liver and tumor/kidney ratios, and significantly superior imaging properties than the initial prototype, NM-324. (Supported by the U. S. Department of Defense grant DAMD17-98-1-8528 and the SPORE in Prostate Cancer grant P50 CA 65968)

We previously described the remarkable capacity of certain radioiodinated phospholipid ether (PLE) analogs to be selectively retained by a variety of rodent and human tumor cell lines [1]. Moreover, this property made it possible to obtain images of these tumors in rabbits, rats and mice using γ-camera scintigraphy. Based

on these and other preliminary results, one of these radioiodinated analogs, 12-(*m*-iodophenyl)dodecyl phosphocholine (NM-324, Figure 1), was approved for pharmacokinetic evaluation in human cancer patients in order to determine whether the results in animals could be confirmed in humans. Although high first pass clearance by the liver compromised the imaging capabilities of NM-324, imaging of the tumors was successful in several patients, and thereby confirmed the potential of radioiodinated PLE analogs for tumor imaging in patients [2].

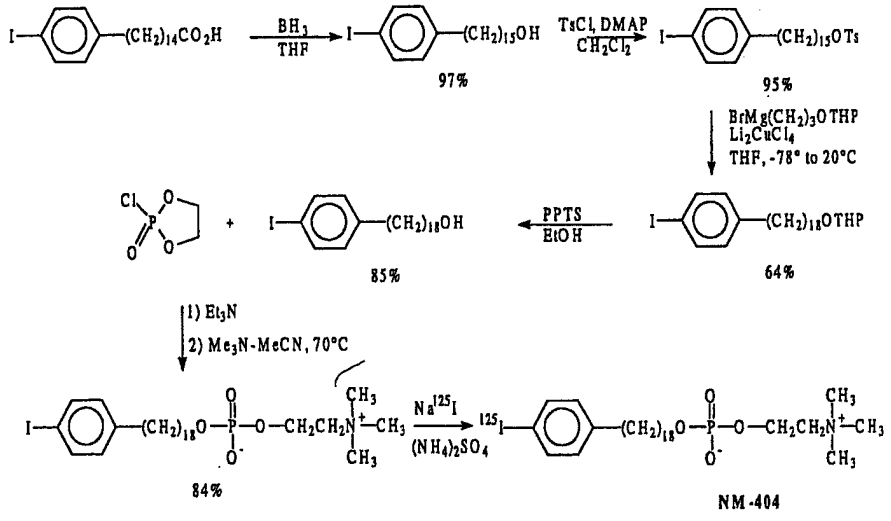


	X	n
NM-324	*m*-^{125}I	12
NM-404	*p*-^{125}I	18

Figure 1. Structures for NM-324 & 404.

In an effort to obtain a more suitable clinical candidate, the present study undertook the synthesis and evaluation of analogs of NM-324 with the aim of improving the tumor retention *vis a vis* the liver and kidneys. Placing the radioiodine in the *para* position and increasing the aliphatic chain length led to NM-404 (Figure 1) which not only increased lipophilicity but also led to the desired properties.

Scheme 1. Synthesis of NM 404



Phosphocholination was performed according to Chandrakumar and Hajdu [3], and radioiodination followed the procedure of Mangner, et al. [4].

Biodistribution analysis (Table 1) and γ-camera imaging was performed in Copenhagen rats bearing Dunning R3327 prostate tumors and in SCID mice bearing human prostate cancer (PC-3). Comparison of NM-324 and 404 over several days revealed that tumor visualization was possible in both instances, but radioactivity was only seen to clear from abdominal organs following administration of NM-404.

Based on these results, NM-404 was selected for further preclinical analysis. The Toxicology Research Center at the University Buffalo found an isotonic solution of stable NM-404 to have no physiologic or pathologic effects in rats or rabbits at a dose 200 times the anticipated human dose. Moreover, the above tissue distribution studies along with those in normal Sprague-Dawley rats predicted that ^{131}I labeled NM-404 could be safely injected in humans with thyroid blocking at a dose of 2 mCi.[5]. Phase I studies in humans are planned.

Table 1. Biodistribution of ^{125}I-NM-404 in male SCID mice bearing PC-3 human prostate cancer xenografts, expressed as Dose/gm ± SEM and Target/Non-target Ratio, (n=4).

	1 DAY	3 DAY	5 DAY	8 DAY
Tissue	% Dose/gm (Tumor/Tissue)	% Dose/gm (Tumor/Tissue)	% Dose/gm (Tumor/Tissue)	% Dose/gm (Tumor/Tissue)
Blood	5.74±0.20 (1.59)	3.10±0.13 (4.24)	3.08±0.09 (5.87)	2.17±0.07 (6.91)
Kidney	4.22±0.14 (2.17)	2.14±0.11 (6.13)	2.28±0.09 (7.92)	1.46±0.04 (10.26)
Liver	3.69±0.21 (2.48)	1.93±0.10 (6.81)	1.63±0.06 (11.07)	1.02±0.06 (14.69)
Lung	5.36±0.33 (1.71)	2.60±0.20 (5.06)	2.27±0.09 (7.97)	1.54±0.06 (9.70)
Muscle	0.79±0.03 (11.50)	0.57±0.04 (22.98)	0.49±0.03 (36.95)	0.40±0.03 (37.33)
Prostate	2.60±0.15 (3.51)	1.40±0.27 (9.40)	1.96±0.25 (9.20)	1.41±0.06 (10.64)
Tumor	9.14±0.69 (1.00)	13.14±0.40 (1.00)	18.06±0.80 (1.00)	14.96±0.63 (1.00)

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